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Effects of liming and fertilization on nematode communities in coniferous forest soils

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Summary. Effects of fertilization and liming on the nematode community structure of the organic and mineral layer of a Scots pine forest were studied three and four years after first applications. Changes in nematode community structure were found for liming only. In the organic layer, in both years significant changes in the composition of the nematode fauna could be shown, whereas this was found for the mineral layer in the fourth year only. Changes in the composition of the nematode fauna were found only when bulk soil pH had increased. Irrespective to soil layer, *Acrobeloides*, *Protorhabditis* and *Eumonhystera* increased, an *Wilsonema* decreased in the limed plots. C-p triangles proved to be a helpful tool in analyzing effects of forest liming and fertilization at nematode community level. Based on our own data on a selection of literature data, it was shown that following liming, taxa with low c-p value (r-strategist *sensu lato*) increased in proportion. This increase appeared after months, and could be demonstrated also after several years. Application of urea also resulted in an increased number of taxa with low c-p value. However, the effects were stronger, appeared within a shorter period of time and seemed to last only for months. Moreover, also the relative number of c-p group 3-5 (K-strategists *sensu lato*) decreased.

Key words: Nematodes, coniferous forest, liming, fertilization, community structure, communities indices

Indroduction

Widespread environmental pollution by atmospherical deposition of among others N- and S-compounds, threatens the ecological functioning of soil ecosystems. In The Netherlands average deposition of NO_x, NH_x and SO_x are 1160, 2400 and 670 $mol \cdot ha^{-1} \cdot y^{-1}$ respectively, with local values of more than 1500, 6000 and 1200 $mol \cdot ha^{-1} \cdot y^{-1}$ respectively (Heij & Schneider 1991). Studies are undertaken to monitor changes in plant and animal communities and functional ecosystem parameters in order to reveal the impact of such pollution. On the other hand experiments are started to study possibilities to counteract the deterioration of the soil environment. The study presented here was part of a multidisciplinary research programme (Harderwijk Forest Fertilization Project) to study whether application of additional nutrients or lime to forest soils, can restore nutrient availability for tree growth, leading ultimately to improved for vitality (Dilz et al. 1990).

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Although these practices aim to aid the recovery of the soil ecosystem in favour to plant growth, they will inevitably lead to disturbance of the actual soil biota community. When studying changes induced in the soil biota community structure (e. g. by forest liming and fertilization) therefore, community indices which are sensitive to environmental disturbance are thought to be a useful tool. To study effects of corrective forest fertilization and liming on soil biota, changes in the nematode community were investigated during two subsequent years. Like other soil fauna, nematode assemblages are thought to reflect the ecological state of the soil environment and because of many supplementary practical and theoretical qualities (e.g. Freckman 1988; Samoiloff 1987) they are considered suitable for monitoring soil quality (Bongers 1990). Forest fertilization and liming experiments in Scandinavia and Central Europe (Bassus 1960, 1967; Huhta et al. 1986; Hyvönen & Persson; Ratajczak et al. 1989) showed effects on total number of nematodes and composition of the nematode community, depending on fertilizer and time of exposure. However, effects on total number of nematodes and trophic structure, proved highly variable. Besides, a comparison of effects at taxa level, is often hampered by differences in species composition between the various experiments. In this study an attempt was made to generalize the observed changes in nematode community composition by using the Maturity Index (Bongers 1990) and c-p triangles (De Goede 1993). The Maturity index is a community index based on life strategies of non-plant parasitic nematodes, and the c-p triangles are graphical presentations derived from this Maturity Index. For this purpose, results were obtained from Forest Fertilization Project Harderwijk and from a selection of literature data.

Material and methods

Site description

The stand, a Scots pine *(Pinus sylvestris)* forest planted in 1960, on a sandy soil, was located in the central part of The Netherlands south east of Harderwijk (52°19′N, 5°40′E). Information on chemical composition of the organic and the mineral layer is given by Dilz et al. (1990) and Hekstra et al. (1990) for 1988 and 1989 respectively. The forest had very little herb layer, with the *P. sylvestris* providing the only roots for root-feeding nematodes; thus the nematode fauna sampled in the litter and upper 10 cm of mineral soil was primarily that of the decomposer food web. Our experiment was part of a larger investigation performed to study the influence of mineral and organic amendments on tree-growth in areas with increased nitrogen deposition. The experimental site was divided in 72 plots, each 22 m × 25 m. Three types of treatments were tested separately, viz. the application of the nutrients (K, Mg, P and Ca), liming, and fertilization with organic manure. Each application was carried out in three replicates which were randomized within each treatment. Based on observed changes in mycoflora (Kuyper & De Vries 1988) and vegetation (Dirkse & Van Dobben 1988), nematode samples were taken from a selection of treatments only. Samples were taken from the K (120 kg K_2O ha^{-1}), Mg (166 kg MgO ha^{-1}), 3 and 9 ton $CaCO_3$ ha^{-1}, and the duck manure (71 kg N ha^{-1}) treatments. K and Mg were applied in three equal parts in spring 1986, 1987 and 1988. In autumn 1985 all limed plots received 3 ton $CaCO_3$ ha^{-1}. After one year the 9 ton $CaCO_3$ ha^{-1} treatment received the remaining 6 ton $CaCO_3$ ha^{-1}. Duck manure was applied in autumn 1985. The duck manure and lime (3 Ca^+ and 9 Ca^+) treatments received additional amounts of K, Mg and P (114 kg P_2O_5 ha^{-1}) to reach the same level of fertilization as in the nutrient treatments. Only the 3 ton $CaCO_3$ ha^{-1} treatments were applied also without these additional nutrients, and are indicated as 3 Ca^-.

Sampling, extraction and counting

On 13 and 14 september, 1988 and 20 november, 1989, respectively three and four years after the first applications, the nematode fauna of the six treatments was sampled. In 1988 samples were taken from the three limed treatments (3 Ca^-, 3 Ca^+ and 9 Ca^+), the duck manure treatment and the control plots. In 1989 the three limed treatments and the Mg and K treatments were sampled including the control plots. All three replicate plots of each treatment were sampled separately. In the central 12 × 15 m from each plot twenty cores (core diameter 30 mm) were taken in a regular pattern over the whole area. The cores were split into organic and mineral layers. For each layer the separate cores were combined into one bulk-sample per plot. In 1988 the mineral layer was sampled to a depth of 10 cm, whereas in 1989 only 0–3 cm mineral layer was sampled. In 1988, three bulk samples per plot were collected from the 3 Ca$^-$ and duck manure treatments to study intra-plot variation. After each bulk sample was mixed carefully, the nematodes were extracted from 25 g fresh weight litter and 100 g fresh mass mineral soil using a modified Oostenbrink elutriation apparatus (Oostenbrink 1960). The litter was soaked one hour in 400 ml water and cut in a blender (5 sec in a Braun MX 32) before extraction (Schouten & Arp 1991). Total numbers of nematodes per sample were estimated by counting 10% of the extracted nematode fauna under a low-magnification dissecting microscope. Afterwards the nematodes were heat-killed and fixed in 4% formalin. The generic composition of each sample was determined under high magnification (400–1000 ×) in subsamples taken from the bulk suspensions. From every single sample at least 100 individuals were identified to genus according to Bongers (1988). From the three replicate samples taken in the 3 Ca$^-$ and duck manure plots, the composition of only one organic layer replicate, but all three mineral soil replicates were analyzed. Water content was determined after 24 h drying at 105 °C. pH(KCl) of the mineral and organic layer was determined by using samples of 10 and 5 g fresh mass respectively in 25 ml 1 N KCl.

Statistics

Changes in population structure were analyzed using correspondence analysis (CA) and canonical correspondence analysis (CCA) (CANOCO, Ter Braak 1988). The analyses were based on relative nematode abundance, and the rare genera (occurring in $<20\%$ of all samples of a horizon) were excluded from the analyses. Differences in generic abundance between the treatments were tested by Wilcoxon non parametric test (Sokal & Rohlf 1981). The taxonomic classification and the classification of the genera into feeding groups follows Bongers (1988) and Bongers et al. (1989) respectively. The Maturity Index was calculated according to Bongers (1990).

Results

Total number of nematodes

In the organic layer, liming tended to reduce the total number of nematodes (Table 1). This reduction was significant only after four years. In the mineral soil no significant differences in total number of nematodes were found. Therefore, relative instead of absolute abundances could be adequately used to describe the effects of the treatments on the nematodes. In 1988 three replicate bulk-samples were collected from each plot of the 3 Ca$^-$ and duck manure treatments. The mean coefficient of variation for the total number of nematodes within the plots was 17.5 for the organic layer, and 21.2 for the mineral layer.

Community analyses

Organic layer: Fig. 1 shows the CCA-plot of the nematode fauna from the organic layer of all plots sampled, with soil pH, treatment and year of sampling as variables. The variation in species composition on the axes is rather low (eigenvalue of first axis is 0.0776) and the first two axes explain 63% of the variance. Despite this low eigenvalue, the species-environment correlation of the first axis is 0.925 and is defined by pH ($r = -0.840$, $P < 0.01$). A Monte Carlo permutation test showed that the pH significantly explained the variation along the first axis ($P \leqq 0.01$). The second axis correlates with the year of sampling ($r = 0.767$, $P < 0.01$). Three and four years after the beginning of fertilization obvious differences in the structure of the nematode fauna of the organic layer were demonstrated between the plots with and without $CaCO_3$ (Fig. 1). No differences in nematode

Table 1. Effect of lime and duck-manure on pH (KCl)[1] and total number of nematodes[2] in the organic layer and mineral soil, three and four years after their first applications

		organic layer						mineral soil				
		lime			manure			lime			manure	
		−		+	−		+	−		+	−	+
pH (KCl)												
1988		3.0	*	5.4	2.9	*	3.2	3.7		3.8	3.4	3.5
1989		2.9	*	5.3	−		−	3.2	*	3.9	−	−
nematode abundance												
1988	mean	3740		2950	3080		2720	1130		1120	770	1000
	s.d.	1005		970	355		495	475		265	195	310
1989	mean	4620	*	3380	−		−	1290		1530	−	−
	s.d.	1025		515				320		315		

[1] pH (KCl) mineral soil 1988 and 1989 respectively taken from 0−10 and 0−3 cm; [2] for organic layer and mineral soil respectively per 25 g fwt and 100 g fwt; * = $p < 0.05$; − not determined

fauna were found between the 3 Ca^+, 3 Ca^- and 9 Ca^+ treatments. However, there was a significant year effect. The canonical correspondence analyses did not show differences between duck manure, Mg and K and their controls. In both years, liming resulted in a significant increase in relative abundance of *Acrobeloides* and *Protorhabditis*, a tendency to increase for *Eumonhystera* and a significant reduction of *Wilsonema* (Fig. 1, Table 2). A

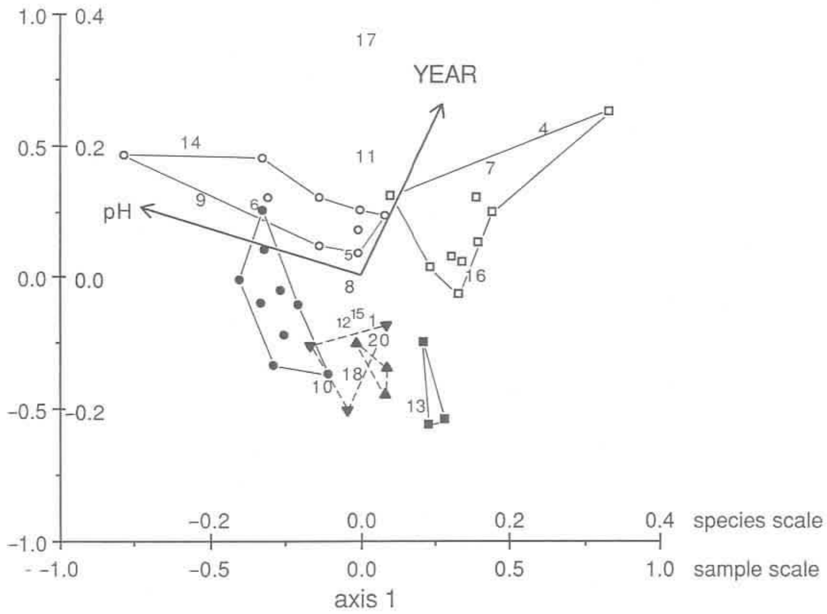

Fig. 1. Ordination (CCA) diagram for axes 1 and 2 based on the nematode fauna of the organic layer, with sites (− lime, ■/□; + lime, ●/○; − duck manure, ▲; + duck manure, ▼; 1988, filled symbols; 1989, open symbols) and taxa (represented by numbers which correspond to table 4) indicated. The main environmental variables are represented by arrows. Note that the axes for sites and taxa have differing scales

Table 2. Effect of lime on the relative numbers (mean %) and frequency (f) of the dominant nematode taxa in the organic layer, three and four years after first fertilization of treatments

Genus	3 years							4 years						
	− lime				+ lime			− lime				+ lime		
	mean	s.d.	f[1]		mean	s.d.	f	mean	s.d.	f		mean	s.d.	f
Filenchus	15.0	3.12	3		10.0	5.04	9	8.7	4.04	9		6.6	4.83	9
Ditylenchus	3.4	1.90	3	*	0.9	1.634	3	9.8	7.15	9		5.5	3.663	9
Aphelenchoides	14.6	4.96	3		20.1	5.87	9	21.3	3.97	9		22.7	7.95	9
Protorhabditis	0.6	1.07	1	*	6.8	4.07	5	3.2	2.19	8	***	8.4	1.66	9
Bunonema	0.0	0.00	0		1.5	2.19	5	6.1	2.20	9	**	3.2	1.65	9
Heterocephalobus	0.6	0.55	2		2.1	2.20	6	2.6	4.11	6		3.7	3.13	9
Acrobeloides	1.9	1.59	3	*	7.5	3.18	9	2.5	1.86	8	**	10.2	5.52	9
Cervidellus	2.5	1.91	3		1.8	2.12	5	0.7	0.81	5		1.6	1.89	6
Drilocephalobus	0.0	0.00	0		0.2	0.63	1	0.8	1.45	3		0.7	1.27	3
Teratocephalus	13.4	4.70	3		12.0	6.56	9	8.2	4.01	9		6.3	4.96	9
Metateratocephalus	5.3	3.73	3	*	1.4	1.82	6	1.4	1.03	8		1.7	1.66	7
Eumonhystera	0.0	0.00	0		3.0	3.44	7	1.3	1.05	8		3.5	3.55	8
Plectus	19.8	8.41	3		21.2	3.84	9	15.6	5.32	9		12.7	4.90	9
Wilsonema	14.1	0.98	3	***	3.6	1.51	9	12.6	6.88	9	*	5.7	4.48	9
Prismatolaimus	0.0	0.00	0		0.1	0.32	1	0.4	0.79	2		0.5	0.63	4
Eudorylaimus	5.3	2.09	3		3.7	1.48	9	2.2	2.28	7		2.6	2.47	8
Tylolaimophorus	1.5	2.67	1		0.5	0.73	4	0.9	0.91	5		1.9	1.25	8

* = $0.05 > p \geq 0.01$; ** = $0.01 > p \geq 0.001$; *** = $p < 0.001$

[1] number of sampling dates on which genus was found in each treatment × replicates; maxima are 3, 9, 9, 9 respectively

Table 3. Effect of duck-manure on the relative numbers (mean %) and frequency (f) of the dominant nematoda taxa in the organic layer and mineral soil, three years after first fertilization

genus	organic layer						mineral soil					
	control			manure			control			manure		
	mean	s.d.	f[1]	mean	s.d.	f	mean	s.d.	f	mean	s.d.	f
Filenchus	8.1	2.78	3	12.5	2.70	3	17.2	5.87	3	17.9	5.66	3
Cephalenchus	0.0	0.00	0	0.0	0.00	0	1.3	1.52	2	* 4.9	1.60	3
Tylenchorhynchus	0.0	0.00	0	0.0	0.00	0	0.0	0.00	0	0.7	0.48	3
Ditylenchus	0.6	0.53	2	0.9	0.93	2	0.6	1.11	1	1.0	0.68	3
Aphelenchoides	20.1	15.29	3	19.1	3.17	3	14.5	8.46	3	18.4	3.10	3
Protorhabditis	2.5	1.92	3	1.9	0.04	3	1.0	1.67	1	1.1	0.96	2
Bunonema	1.6	2.72	1	1.9	0.94	3	0.0	0.00	0	0.0	0.00	0
Heterocephalobus	3.8	5.00	2	2.6	4.44	1	0.3	0.56	1	0.7	0.18	3
Acrobeloides	3.4	1.45	3	4.1	1.53	3	9.4	3.87	3	11.2	3.57	3
Cervidellus	2.8	2.48	2	3.1	2.15	3	24.5	9.56	3	14.5	6.62	3
Drilocephalobus	0.0	0.00	0	0.6	1.07	1	5.6	6.61	2	6.0	1.38	3
Teratocephalus	6.2	4.61	3	11.7	5.32	3	5.5	3.88	3	7.7	3.03	3
Metateratocephalus	4.7	0.98	3	3.4	3.53	3	0.6	1.11	1	0.1	0.17	1
Eumonhystera	0.3	0.53	1	0.6	1.11	1	0.0	0.00	0	0.3	0.36	2
Plectus	20.0	2.16	3	24.1	3.83	3	3.5	2.76	3	4.2	1.45	3
Wilsonema	9.3	4.78	3	4.1	3.24	3	2.9	0.05	3	2.6	0.37	3
Prismatolaimus	0.0	0.00	0	0.0	0.00	0	0.0	0.00	0	0.3	0.31	2
Eudorylaimus	4.0	1.39	3	4.4	3.48	3	1.9	2.54	2	0.4	0.17	3
Tylolaimophorus	3.7	2.52	3	1.6	0.52	3	5.5	3.50	3	2.3	0.70	3
Steinernema	0.0	0.00	0	0.0	0.00	0	1.6	1.51	2	1.3	0.61	3

[1] number of sampling dates on which genus was found in each treatment × replicates; maxima are 3, 3, 3, 3 respectively; * = $p < 0.05$

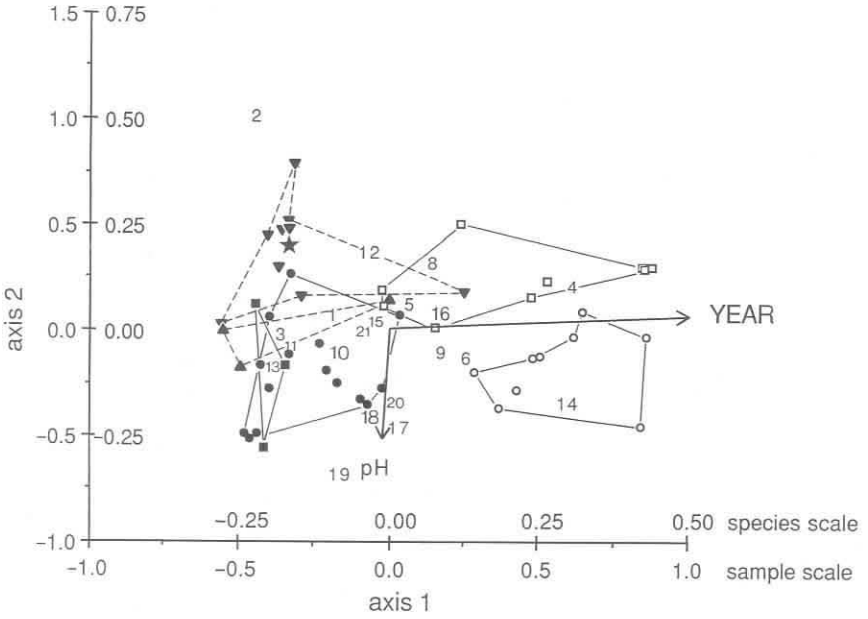

Fig. 2. Ordination (CCA) diagram for axes 1 and 2 based on the nematode fauna of the mineral soil. Taxa numbers correspond to table 4. See fig 1 for explanation of symbols

significantly lower relative abundance of *Ditylenchus* and *Metateratocephalus* was found three years after Ca$^-$ application, whereas *Bunonema* had a significantly lower relative abundance after four years only (Table 2). In the organic layer, no significant effect of application of duck manure on any nematode taxon was found (Table 3).

Mineral layer: As compared with the organic layer, the nematode community structure in the mineral layer showed less effects of the treatments (Fig. 2). The first CCA axis had an eigenvalue of 0.138 and correlated best with the year of sampling ($r = 0.861$, $P < 0.01$). The second axis (eigenvalue 0.063) had a species-environment correlation of only 0.836. This axis correlated best with pH ($r = -0.589$, $P < 0.01$) and application of duck manure ($r = 0.776$, $P < 0.01$). Thus, most of the variation in the composition of the nematode fauna was correlated with the year of sampling. In the plots without $CaCO_3$ application, *Metateratocephalus*, *Tylenchorhynchus*, *Drilocephalobus*, *Cervidellus* and *Filenchus* had lowest frequency and/or relative abundance after four years (Fig. 2, Table 4). The opposite was true for *Ditylenchus*, *Eumonhystera*, *Acrobeloides*, *Wilsonema* and *Aphelenchoides*. After three years, community analyses of the nematode fauna in the mineral layer did not show effects of liming (Fig. 2), although *Acrobeloides* and *Drilocephalobus* had significant higher, and *Plectus* significant lower abundances in the limed plots (Table 4). The changes in the composition of the nematode fauna of the organic layer were significantly correlated with pH. However, up to four years after liming no change in pH was found in the 0–10 cm mineral soil (Table 1). Since the lime was applied on top of the organic layer, an increase of pH of the mineral soil is likely to occur first in the upper part of mineral soil. Therefore, the nematode fauna of the first 3 cm mineral soil was sampled in the fourth year. Table 1 shows that pH increased 0.7 units in the plots which received $CaCO_3$. These changes in pH were correlated with changes in the composition of the nematode fauna (Fig. 2). The genera *Acrobeloides* and *Eumonhystera* had significantly higher relative abundances in the limed plots, whereas *Wilsonema*, *Teratocephalus*, *Aphelenchoides* and *Steinernema* had significantly lower ones (Table 4). In the plots fertilized with duck manure the only significant effects found were an increase of *Cephalenchus* and a decrease of *Tylolaimophorus* (Table 3).

Table 4. Effect of $CaCO_3$ on the relative numbers (mean %) and frequency f[1] of the dominant nematode taxa in the mineral soil[2], three and four years after first applications of treatments

Genus	C	T	3 years							4 years						
			− lime				+ lime			− lime				+ lime		
			mean	s.d.	f		mean	s.d.	f	mean	s.d.	f		mean	s.d.	f
1 *Filenchus*	−	P	22.2	4.39	3		16.8	7.24	9	12.8	9.32	9		6.5	5.09	9
2 *Cephalenchus*	−	P	0.0	0.00	0		0.1	0.20	1	0.0	0.00	0		0.1	5.09	9
3 *Tylenchorhynchus*	−	P	2.1	1.93	2		0.4	0.58	4	0.1	0.28	1		0.0	0.00	0
4 *Ditylenchus*	2	H	0.9	0.93	2		0.5	1.04	2	12.5	11.33	8		8.8	4.51	9
5 *Aphelenchoides*	2	H	15.8	8.55	3		18.4	8.98	9	24.6	4.32	9	*	18.5	5.21	9
6 *Protorhabditis*	1	B	0.9	0.90	1		0.9	0.73	4	1.5	1.86	7		3.8	1.82	9
7 *Bunonema*	1	B	0.0	0.00	0		0.0	0.00	0	0.0	0.00	0		0.1	0.27	2
8 *Heterocephalobus*	2	B	0.6	1.08	1		0.1	0.32	1	0.3	0.71	2		0.9	1.33	5
9 *Acrobeloides*	2	B	2.8	2.49	3	*	12.4	5.21	9	11.6	6.62	9	***	24.5	5.67	9
10 *Cervidellus*	2	B	23.8	6.06	3		18.0	4.29	9	9.9	5.39	9		9.9	2.90	9
11 *Drilocephalobus*	2	B	3.1	1.44	3	*	8.5	4.08	9	0.9	1.11	5		1.1	1.05	6
12 *Teratocephalus*	3	B	2.1	1.05	3		3.5	2.53	7	6.6	5.03	9	**	1.9	1.73	8
13 *Metateratocephalus*	3	B	1.5	1.42	2		0.5	0.66	5	0.0	0.00	0		0.1	0.43	1
14 *Eumonhystera*	1	B	0.0	0.00	0		0.3	0.62	3	0.7	0.63	6	*	2.8	2.61	9
15 *Plectus*	2	B	5.5	3.16	3	*	2.7	1.34	9	3.2	2.15	9		3.0	1.82	9
16 *Wilsonema*	2	B	2.8	0.94	3		3.3	1.29	9	7.4	2.48	9	*	4.7	2.63	9
17 *Prismatolaimus*	3	B	0.6	0.53	2		2.7	2.41	8	0.5	0.99	3		1.9	1.88	8
18 *Eudorylaimus*	4	O	1.2	1.37	2		1.4	1.13	6	0.5	0.74	4		1.2	0.65	8
19 *Tylencholaimus*	4	H	0.9	0.93	2		0.4	0.68	3	0.0	0.00	0		0.3	0.56	3
20 *Tylolaimophorus*	3	H	11.2	14.54	3		6.2	4.03	8	4.4	2.48	8		7.5	4.71	9
21 *Steinernema*	−	I	0.9	1.62	1		1.7	1.48	8	1.7	1.28	7	*	0.5	0.59	5

[1] number of sampling dates on which genus was found in each treatment × replicates; maxima are 3, 9, 9, 9 respectively; [2] samples taken from 0−10 and 0−3 cm for respectively 1988 and 1989; C: c-p value of genus used to calculate the MI, plant feeding and insect parasitic genera were excluded from the calculation of the MI; T: trophic group, P plant feeding, H hyphal feeding, B bacterial feeding, O omnivores, I insect parasites; significant differences between relative abundances of + lime and − lime plots are shown for each year separately: * = $0.05 > p \geqq 0.01$; ** = $0.01 > p \geqq 0.001$; *** = $p < 0.001$

Table 5. Effect of $CaCO_3$ and duck-manure on trophic group distribution, % Secernentia, % Rhabditida, Maturity Index (MI) and c-p value group distribution in the organic layer and mineral soil, three and four years after the first applications of treatments

	organic layer						mineral soil				
	1988			1989			1988		1989		
	− lime		+ lime	−lime		+ lime	−lime	+ lime	−lime		+ lime
	n = 3		n = 9	n = 9		n = 9	n = 3	n = 9	n = 9		n = 9
plant feeding	15.3		10.4	9.3		7.2	24.6	18.0	12.9		6.9
hyphal feeding	19.6		21.6	32.2		30.1	28.8	25.5	41.4		35.2
bacterial feeding	58.2		61.5	55.7		58.8	44.4	53.3	43.0	**	55.2
omnivores	5.3		3.7	2.2		2.6	1.2	1.5	0.5	*	1.2
insect parasites	0.0		0.0	0.2		0.0	0.9	1.7	1.7		0.8
% Secernentia	58.8		66.6	66.4	**	72.2	75.9	80.9	80.9		76.6
% Rhabditida	24.7	*	34.1	25.9	**	36.3	34.9	44.0	30.9	*	42.5
MI	2.36	***	2.12	2.06		2.01	2.26	2.20	2.13		2.09
c-p 1	0.7	***	13.0	12.1	*	16.5	1.2	1.6	2.6	***	7.4
c-p 2	68.5		66.2	73.1		68.7	74.3	79.5	83.0		77.9
c-p 3	24.4		16.5	12.0		11.8	21.6	16.4	13.5		12.8
c-p 4	6.4		4.4	2.8		3.0	2.8	2.4	0.9	*	17
c-p 5	0.0		0.0	0.0		0.0	0.0	0.1	0.0		0.1

* = $0.05 > p \geqq 0.01$; ** = $0.01 > p \geqq 0.001$; *** = $p < 0.001$

Trophic structure

Bacterial feeding nematodes were dominant both in the organic and mineral layer (Table 5). Predatory nematodes were not found. The only significant difference in trophic structure between the plots due to liming, was an increased number of omnivores and bacterial feeding nematodes in the mineral layer after four years. The same trend, although not significant, was already apparent in the previous year. In the organic layer as well as in the mineral soil hyphal feeding nematodes made up a larger part of the nematoda fauna in 1989 compared with 1988, this at the expence of the plant feeding nematodes. This was mainly due to shifts in relative abundance of *Filenchus* and *Ditylenchus*. Although significant effects of liming on bacterial feeding taxa were found, no differences were found when the bacterial feeding nematodes were taken as a group. No significant changes in trophic structure could be detected in response to the application of duck manure.

Community indices

The percentage Secernentia (= Tylenchida + Rhabditida) in the organic layer increased from 59 in 1988 to 66 in 1989, in the untreated control plots (Table 5). Liming resulted after four years in a further and significant increase up to 72%. The percentage Secernentia in the mineral layer was higher than in the organic layer, but was not significantly affected by liming. In both years a significant higher percentage Rhabditida was found in the organic layer of the limed plots. In the mineral soil a significant increase was found only after four years. In the organic layer of the limed plots a significant reduction of the MI was observed after three years, as compared to the plots without lime (Table 5). The MI in the plots without lime decreased from 2.36 in 1988 to 2.06 in the next year, and the differences between plots with and without lime had then disappeared. The changes in MI were due to an increase of nematode taxa with a c-p value 1 (Table 5). In the mineral layer no differences in MI between the plots with and without lime were found, although after four years the nematode taxa with c-p value 1 increased significantly in the limed plots. However, at the same time, c-p value group 4 increased in the limed plots as well, resulting in similar MI values. The results obtained from the MI and the percentage Rhabditida largely parallel, which can be explained by the fact that all families with c-p value 1 (except the Monhysteridae) belong to the Rhabditida. The only effect, although small, of application of duck manure was a significant decrease of the MI of the mineral layer, from 2.22 to 2.14.

C-p triangles

Harderwijk Forest Fertilization Project: In Fig. 3 the c-p triangles for the Harderwijk forest are given. In the graphs the proportion of c-p group 1, c-p group 2, and the sum of the c-p groups 3, 4 and 5 are given on a side of the triangle. In both years, in the organic layer liming resulted in increased numbers of c-p group 1, whereas the relative abundance of c-p group 3 – 5 was not altered (Fig. 3A and B). After three years, c-p group 1 composed only a relative insignificant part of the nematode fauna of the mineral layer (Fig. 3C). One year later the proportion of c-p group 1 increased in the $CaCO_3$ treatments, but remained low in the control (Fig. 3D).

Other forest fertilization projects: Fig. 4 shows c-p triangles of nematode assemblages in a number of forest fertilization, liming and acidification experiments in Scandinavia and Central Europe (Hyvönen & Huhta 1989; Hyvönen & Persson 1990; Bassus 1960; Schouten unpubl.). The selection of experiments is confined to field studies of coniferous forests, in which full taxa lists are given. The fertilization and liming experiments can be divided into application of urea, ash and mineral calcium compounds ($CaCO_3$, CaO and $Ca(OH)_2$), all three resulting in an increased soil pH. In the acidification experiments at Fexboda and

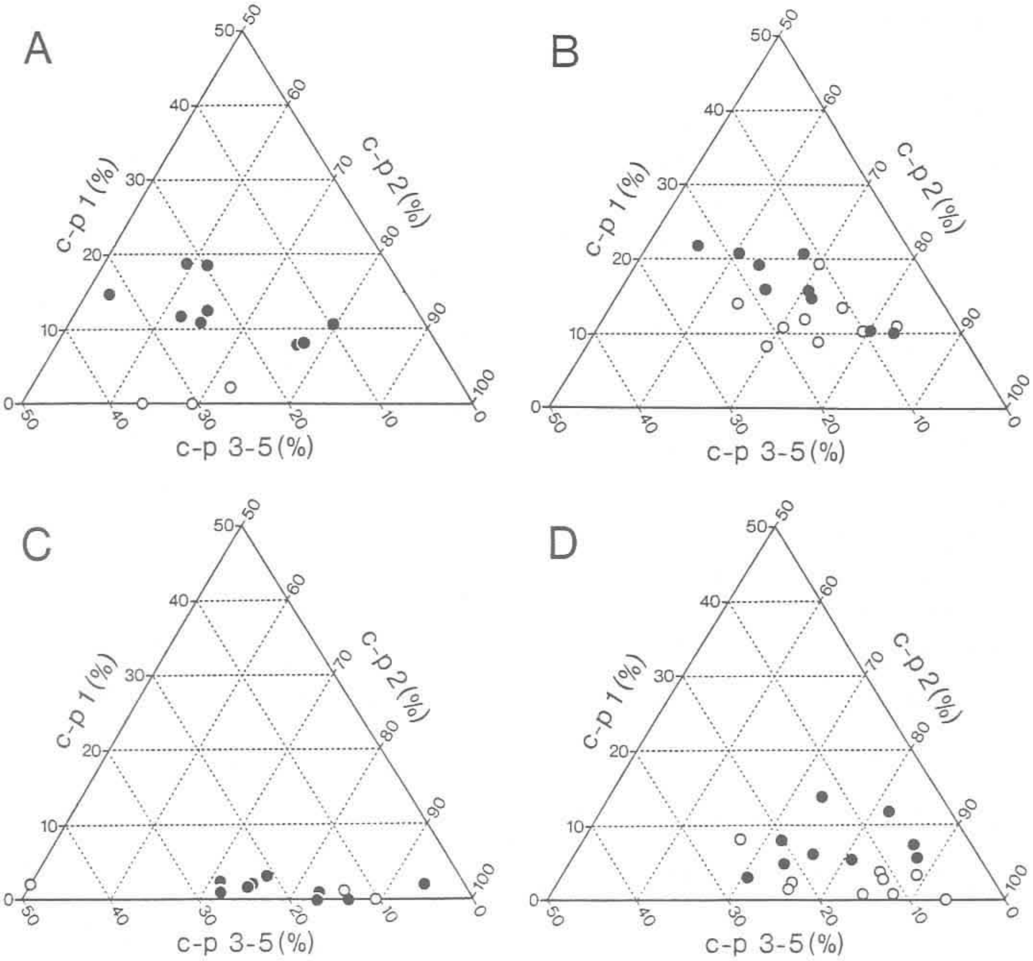

Fig. 3. C-P triangles for the organic layer 1988 (A) and 1989 (B) and for the mineral soil 1988 (C) and 1989 (D) of Harderwijk forest (− lime: ○, + lime: ●). Note that only a section of the total triangle is shown

Norrliden, H_2SO_4 was used to lower soil pH. In samples taken within the first three months after liming with calcium compounds no shifts in c-p value group distribution occurred (Fig. 4A and F). However, four, seven and twelve months after liming the proportion of c-p group 1 was higher (Fig. 4F). A comparable increase was also found in other experiments two and six years after liming (Fig. 4C respectively D).

In Tiefensee (Fig. 4E) and Norrliden (Fig. 4C), respectively one and twelve years after liming, the proportion of c-p group 3−5 in the limed plots was higher than in the corresponding controls.

Fertilization with urea resulted in a clear short-term shift in c-p value group distribution (Fig. 4A and B). One month after the start of the urea fertilization the proportion of c-p group 1 increased to 50% and the proportion of c-p group 3−5 dropped below 10% (Fig. 4A). After a further increase to 67% in the next month, c-p group 1 subsequently decreased to a level of 25% four months after the start of the experiment. No recovery of c-p group 3−5 could be observed. During this four months period the MI of the urea treated plots was respectively 1.55, 1.36, 1.79 and 1.84, which is less than in the control (average MI is 2.20). The second year after application of urea, another experiment showed

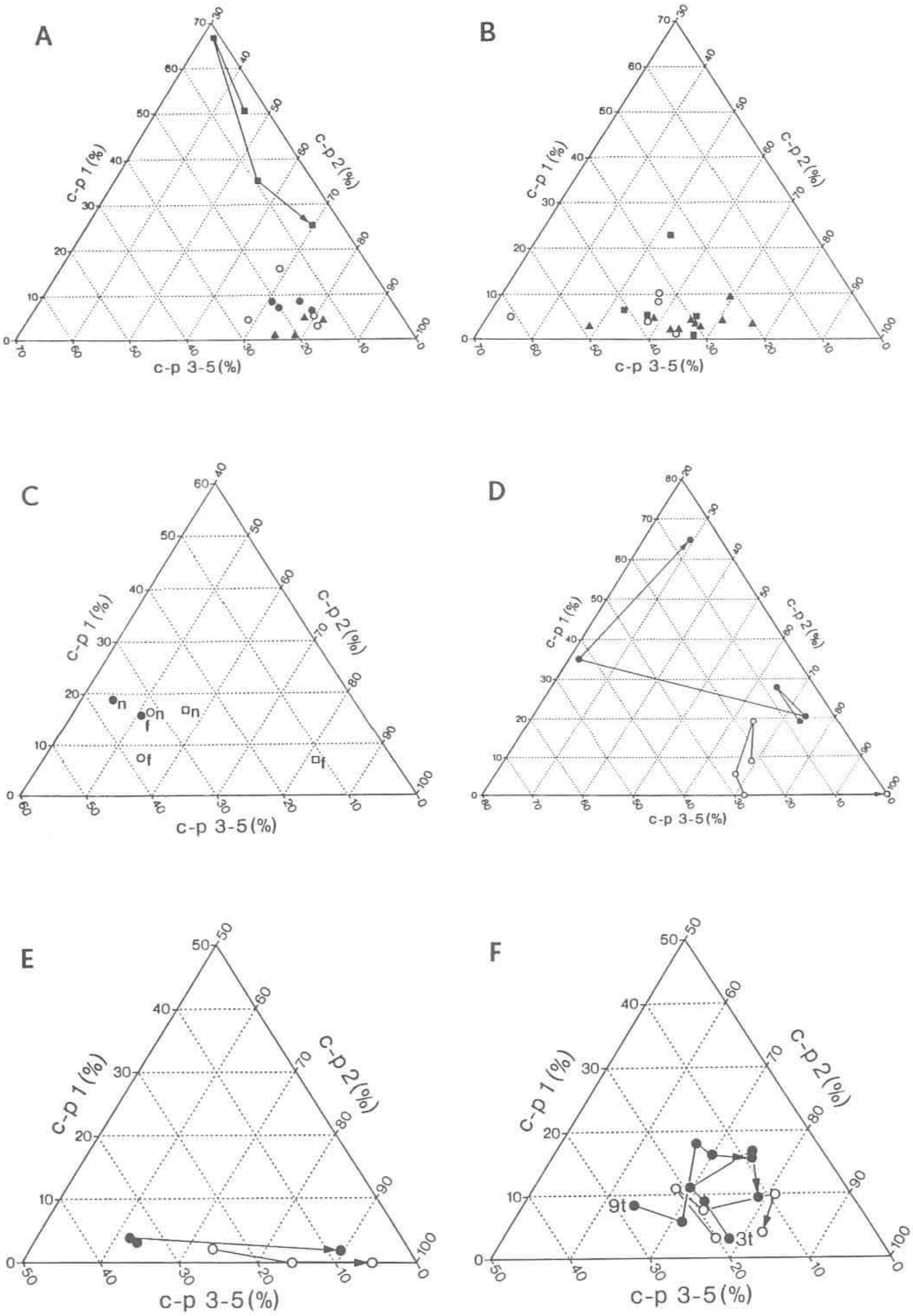

Fig. 4 (see page 13)

no differences in c-p value group distribution (Fig. 4B).

The first year after application of ashes no differences in c-p value group distribution were found (Fig. 4A), whereas after one year a decrease of c-p group 3 – 5 possibly occurs (Fig. 4B). Artificial acidification also resulted in a relative decrease of c-p group 3 – 5 (Fig. 4C) which was most obvious in Fexboda where the final application of H_2SO_4 was 6 months before sampling. Seven years after final artificial acidification only a relatively small decrease of c-p group 3 – 5 was visible (Norrliden), possibly indicating some recovery.

From an analyses of literature data and our own data, it can be concluded that the c-p value group distribution can be helpful in analyzing effects of disturbance like forest fertilization. Fertilization with urea leads to dramatic short term (weeks) shifts in the composition of the nematode fauna (increase of c-p group 1 and decrease of c-p group 3 – 5), which seem to last only for several months. Shifts in the composition of the nematode fauna due to liming with calcium compounds (increase of c-p group 1) appear after months, and can be still demonstrated after several years. Artificial acidification is found to lead to a relative decrease of c-p group 3 – 5.

Discussion

Effects of lime on nematode fauna

Forest liming is thought to result into increased activity of bacterial populations (reviewed by Persson 1988) and therefore changes within bacterial feeding nematode population can be expected. Three and four years after the first application of lime at Harderwijk, no increase of the bacterial feeding nematode population as a whole was found in the organic layer. However, the proportions of the bacterial feeders *Acrobeloides*, *Protorhabditis* and *Eumonhystera* were increased, and their absolute numbers were 2.3 to 5.5 times higher than in the plots which did not receive lime. An increase in density of *Acrobeloides*, species of the Rhabditidae and, if present *Eumonhystera*, is found in most forest fertilization experiments where fertilizers caused decreasing acidity (Hyvönen & Huhta 1989; Bassus 1960, 1967; Ratajczak et al. 1989). The Rhabditidae and *Eumonhystera* are considered to be r-strategists (*sensu lato*; c-p value 1) characterized *inter alia* by short generation times and numerous progeny (Bongers 1990). Their occurrence, as well as that of *Acrobeloides*, is found to be positively correlated with the productivity of bacteria populations (Schiemer 1983; Sohlenius 1973).

◄

Fig. 4. C-P triangles for forest fertilization experiments A: Scots pine forest at Ruotsinkyla (Hyvönen & Huhta 1989; sampling at monthly intervals, 1 – 4 months after first fertilization), B: Scots pine forest at Tammela (Hyvönen & Huhta 1989; sampling at monthly intervals, 12 – 16 months after first fertilization), C: Norway spruce forest at Fexboda (sub-script "f") and Scots pine forest at Norrliden (subscript "n") (Hyvönen & Persson 1990; sampling at respectively two and twelve years after first applications[1]), D: spruce forest at Hermsdorf (Bassus 1960; sampling during the 6th and 7th year after liming), E: spruce forest at Tiefensee (Bassus 1960; sampling at III, V and X, one year after liming), F: Scots pine forest at De Peel (Schouten, unpubl.; sampling at one week before and six weeks respectively 4, 7 and 12 months after liming).

Applications are indicating as ■: urea compounds, ●: Ca compounds, ▲: ashes, □: H_2SO_4, ○: controles. In some triangles, time-series are connected with solid lines, in which the arrow indicates the last sampling occasion. In figure F the applied dose of lime (3 or 9 ton ha^{-1}) is indicated at the beginning of each time-series of samples, and figures shown are means of two replicate fields

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[1] Acidification was repeated yearly until the year of sampling at Fexboda, and until 7 years before sampling at Norrliden

In the same experiment at Hardewijk Kuyper & De Vries (1990) found a decreased diversity and abundance of litter decomposing fungi in the limed plots. The total number of hyphal feeding nematodes was not significantly influenced by liming. However, the relative abundance of the hyphal feeding genus *Ditylenchus* was lower in the limed plots. It should be noticed that in some cases it appeared to be difficult to distinguish juvenile plant-parasitic *Filenchus* species (*Filenchus* cf *ditissimus*; trophic group classification according to Yeates et al. (in press)) from juveniles of morphologically very similar *Ditylenchus* species. In addition, four years after liming (i.e. the first year that significant differences in the composition of the nematode fauna were observed in the mineral soil due to liming) the relative abundance of the dominant hyphal feeder *Aphelenchoides* was significantly reduced in the mineral soil of the limed plots. These findings support the general observation that a decreased acidity results in conditions favouring bacteria relative to fungi (Alexander 1977; Swift et al. 1979; Persson 1988). Huhta et al. (1986) concluded that it seems to be plausible that the decrease of acidity alone is enough to explain the changes in soil fauna composition. Our results do not refute this idea; changes in the nematode taxa given above are found after liming only, and only when bulk soil pH has been increased. Although no species were found to become extinct after bulk soil pH increased by two or more units, the diversity found here might not be the historical potential richness of the nematode fauna. Since no pre-pollution surveys on Dutch nematode communities exist, the potential richness of the nematode fauna in these environments is unknown.

Maturity Index and c-p triangle

Interpretation of differences in relative distribution of the c-p groups for the above mentioned experiments, revealed changes and trends in situations where no change in MI could be found. Most of the MIs of the coniferous forests in which fertilization experiments were carried out, lay between 1.9 and 2.5. Within this range of maturity indices an arbitrary MI can come about by a wide range of different c-p value group distributions (De Goede 1993). For instance, two samples with comparable MI may differ significantly in relative distribution of r- and K-strategists *(sensu lato)*. Reduction of a nematode taxa list into a c-p value group distribution based on autecological characteristics of the nematode species, still reveals effects of environmental disturbances on the nematode fauna. But a further reduction of this information into a MI can thus obscure more or less gradual effects on nematode communities. Acute effects like those caused by application of urea, which result in a nematode community with c-p group 1 and c-p group $3-5$ of respectively >20 and $<10\%$, will lead to maturity indices $\leqq 1.8$. In general, such low maturity indices indicate environmental disturbances.

Response time of the nematode fauna

Although both fertilization with urea or lime seem to result in an increased proportion of nematode taxa with low c-p values, the times of first response, intensity, and duration of response, appear to differ significantly. Application of urea results in strong, short-term changes in the nematode community (Huhta et al. 1986), whereas effects of liming with calcareous compounds are observed not until several months, but they seem to last for several years. Relatively rapid shifts in pH, enrichment with nitrogen or input of dead organic material (e.g. because of die back of mosses) can be responsible for the effects of urea (Huhta et al. 1986). The gradual shifts in nematode community composition after liming with calcareous compounds agree with the relatively low solubility rate of calcium carbonate. In contrast to urea the increase in soil pH lasts for decades and effects on pH in the mineral soil are, compared to the organic layer, delayed (Nihlgård et al. 1988). The same was found for effects on the composition of the nematode fauna in our experiment and in similar expriments in Dutch Scots pine forests (Schouten et al., in prep). In our experiment, effects due to differences in sampling depth between the years cannot be excluded, but are thought to be of minor importance. First changes in nematode fauna composition are expected initially to occur in the top centimetres of the mineral soil. As nematode population density decreases with soil depth, changes in nematode fauna composition three years after liming, when 0 – 10 instead of 0 – 3 mineral soil was sampled, should have been noticed.

State of the nematode fauna

Comparison of the average c-p group 4 – 5 scores, i.e. the proportion of extreme K-strategists *(sensu lato)*, of the control plots from two fertilized Scots pine forests from The Netherlands (this study; Schouten et al., in prep) with five Scots pine forets from Scandinavia (Sohlenius & Wasilewska 1984; Hyvönen & Huhta 1989; Hyvönen & Persson 1990), shows lower values for the Dutch forests: 0.7 – 5.8% respectively 11.7 – 24.4%. Comparable low values (2.1 – 8.1%) are found also in Dutch Scots pine forests at other locations (own observations). In addition, experiments studying effects of artificial acidification showed a decrease or disappearance of especially nematode taxa with high c-p values (Fig. 4C; Ratajczak et al. 1989; Schouten & Van der Brugge 1989). If these taxa with high c-p values are part of the potential fauna, the absence of these taxa possibly coincides with the decline of Dutch forests on sandy soils. Although in Harderwijk, no new nematode taxa were noticed in the limed plots, within four years of liming. Floristic- and myco-sociological studies on the other hand did record higher plant and fungal species in the limed treatments of Harderwijk forest, which were not found in the non-limed plots (Dirkse & Van Dobben 1988; Kuyper & De Vries 1990).

Conclusions

- Three and four years after liming, the total number of nematodes tended to be reduced in the organic layer, whereas no significant effects were found in the mineral layer.
- In the organic layer, differences in the structure of the nematode fauna were demonstrated three and four years after liming. In the mineral layer such differences were found only four years after liming. These differences only occurred when bulk soil pH(KCl) had increased.
- Liming resulted at all times, irrespective of soil layer, in a relative increase of *Acrobeloides*, *Protorhabditis* and *Eumonhystera*, and a relative decrease of *Wilsonema*.
- From the community parameters tested, only percentage Rhabditida and c-p group 1 responded significantly at all these times when changes in nematode community structure due to liming were found.
- Although significant effects of liming on bacterial feeding taxa were found, no differences were found when the bacterial feeding nematodes were taken as a group.
- C-P triangles prove to be a helpful technique in studying disturbances of the nematode fauna in soil ecosystems.
- Liming and fertilization with urea result in increased proportions of nematode taxa with c-p value 1.

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